NTP
National Toxicology Program

Utility of Genetically Modified Models (GMMs) in NTP Cancer Hazard Identification

NTP Board of Scientific Counselors
Technical Reports Review Subcommittee
August 28, 2006

Outline:

- History of development of GMMs at NIEHS and NTP
- Scientific and Technical Report Reviews
  - First Board of Scientific Counselors, Feb. 1998
    - First TRR Subcommittee, Sept. 2002
  - Second Board of Scientific Counselors, Sept. 2002
    - NTP Workshop, Feb. 2003
    - Second TRR Subcommittee, May 2003
  - Current TRR Subcommittee, Aug. 2006
- Update sensitivity/specificity data for GMMs
- Can GMMs be used for risk assessment?
- Ongoing studies
- Proposal for use of GMMs in cancer hazard identification

History of Use of GMMs for Cancer Studies at the NIEHS

- Tennant et al.
  - MMTV driven v-Ha-ras, c-myc, c-neu mammary tumor models of Leder (1993)
  - v-Ha-ras Tg.AC (ζ- globin promoter) skin tumor model of Leder (1993 -)

- French et al.
  - p53 +/- knock out mouse of Donehower (1997 -)

- Maronpot et al.
  - Tg rasH2 (c-Ha-ras expression) developed by Katsuki (2000 -)
  - TRAMP (Pb-tag) prostate cancer model

- Rao et al.
  - MMTV driven v-Ha-ras, c-myc, c-neu mammary models (prevention)

- Mahler et al.
  - PIM1 lymphoma model

History of Use of GMMs for Cancer Studies at the NIEHS

- Eastin et al.
  - Tg.AC and p53+/- studies of genotoxic and non genotoxic human and rodent carcinogens and noncarcinogens *Tox Path* 26:461-473 1998
  - ILSI/HESI ACT collaboration Tg.AC, *Tox Path* 29 (Suppl.) 2001
- Dunnick et al.
  - p53 +/- (1997 -) phenolphthalein, methylphenidate other models- APC, p16/p19 +/-
  - French et al. p53 +/- (1997 -) phenolphthalein molecular analysis
- Spalding et al.
  - p53+/- and Tg.AC prospective studies on nine bioassay chemicals *Tox Sci*. 53:213-223, 2000

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First Board of Scientific Counselors Review

- NTP Board of Scientific Counselors, Feb. 1998
  - Conditional acceptance
    - p53+/- accepted, Tg.AC questioned
    - Noted lack of dose response information
    - Lack of understanding of “misses”

- Urged development of specific tumor site models and continued effort on these and other models for general carcinogen screens

First Technical Report Subcommittee Review

- NTP Board of Scientific Counselors Technical Reports Subcommittee, Sept. 2002
  - Review of Tg.AC studies of Pentaerythritol triacrylate and Trimethylolpropane triacrylate
  - Reports proposed for TR series- “blue books”
  - Question posed
    - In your opinion, is there sufficient scientific evidence using this model to evaluate the potential carcinogenicity of each compound? If not what steps should the NTP take next?
  - Discussion points
    - Dose selection
    - Verify Tg activation?
    - Relationship between neoplasms and non neoplastic lesions
    - Statistics-incidence, multiplicity, both?
    - What is an adequate negative study?
    - What does a tumorigenic outcome mean?
- Subcommittee rejected proposed conclusion of “clear evidence of carcinogenic activity”
- Suggested that more appropriate, model-specific descriptive language be developed

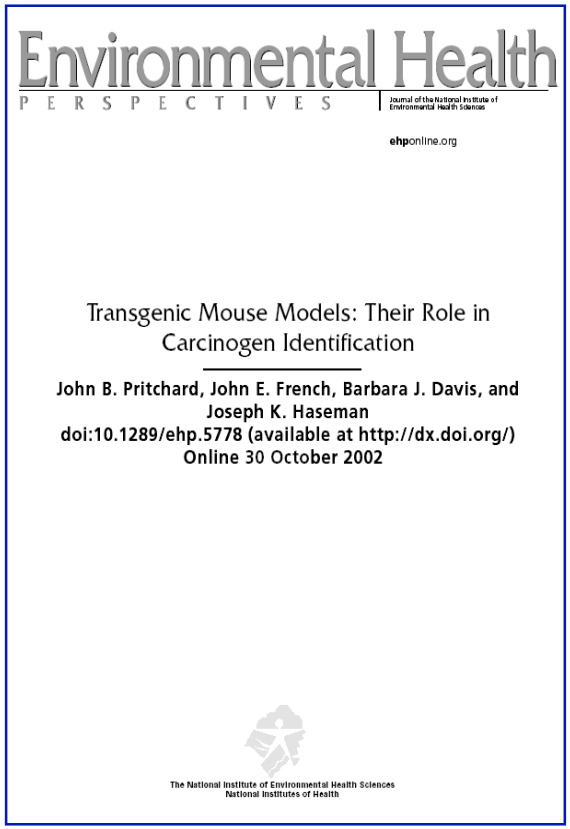
Second Board of Scientific Counselors Review

NTP Board of Scientific Counselors and Ad hoc Experts- Sept. 2002

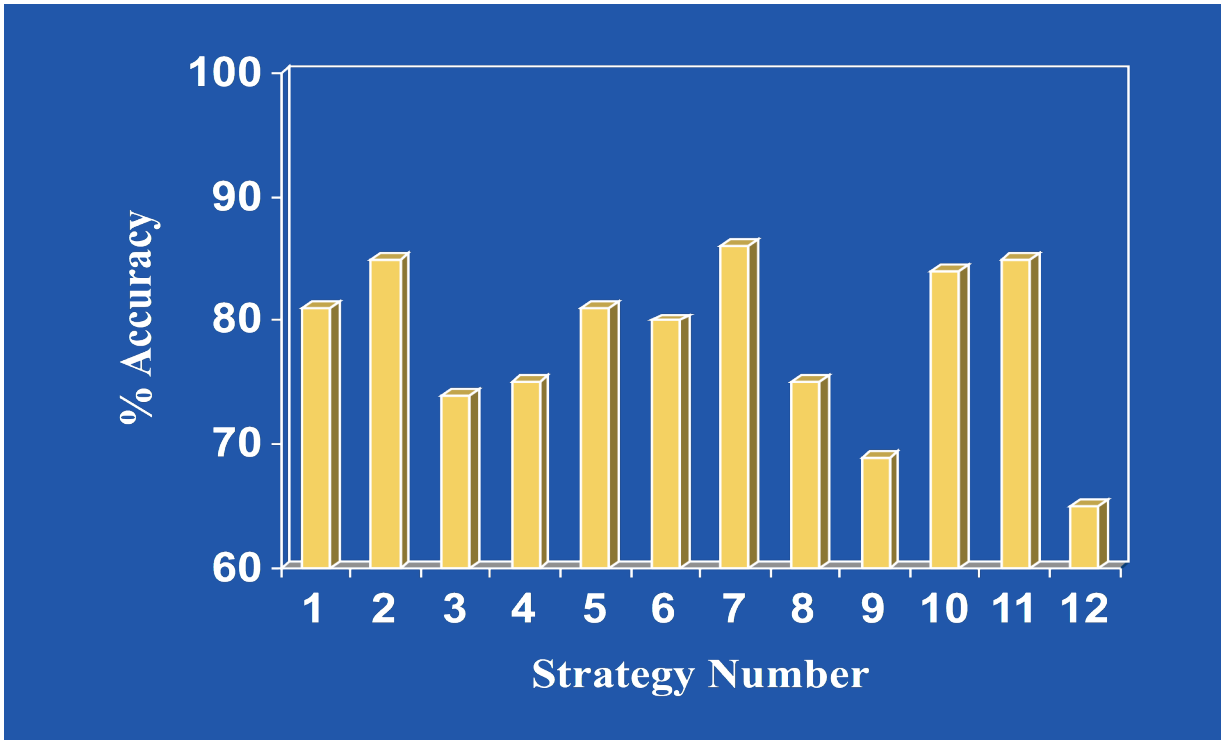
- Asked to review status of scientific acceptance of GMM results in light of the TRR Subcommittee recommendations on the acrylates
- Presented state of understanding of the Tg.AC, p53, and rasH2 models
- Considered findings of ILSI coordinated program on Alternative Models for Carcinogenicity Assessment
- Considered review of collected published results to date by Pritchard, et al. (included most of the ILSI findings)
- Considered several testing strategies for optimal sensitivity and specificity

Second Board of Scientific Counselors Review

- Pritchard et al. evaluation
  - Concordance of selected model results with IARC and ROC carcinogen lists
  - Design and analysis issues



Transgenic Model Performance



1 = Trp53+/-

2 = Trp53+/- G only

3 = Tg.AC

4 = RasH2

5 = p53-G/Ras-N

6 = p53-G/Ras-All

7 = p53-G/Tg.AC-N

8 = p53-G/TgAC-All

9 = Rodent Bioassay

10 = Rat Bioassay Plus Tg.AC-N or Trp53-G

11 = Rat Bioassay Plus RasH2-N or Trp53-G

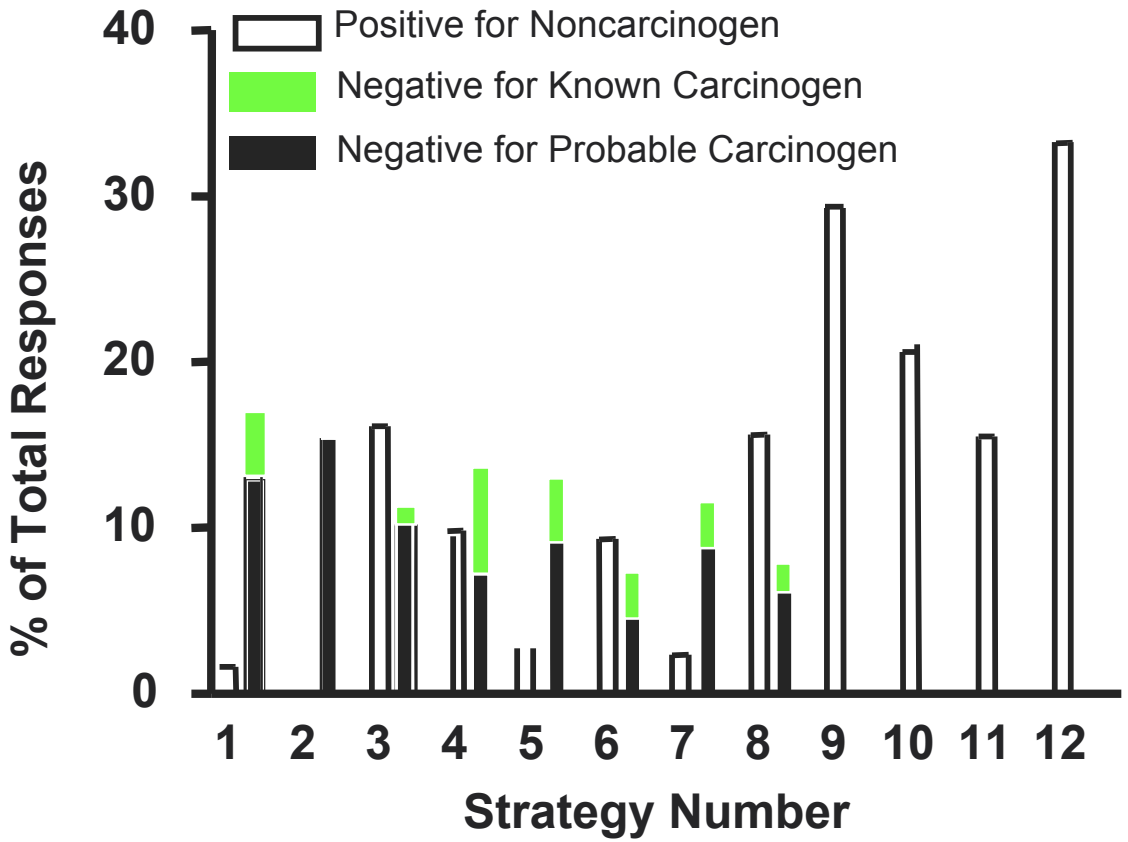
12 = Rat Bioassay Plus Genotoxicity

% Accuracy = Positive findings for IARC/ROC Known/ Suspected Carcinogens plus Negative for IARC/ROC Non-Listings

G = Genotoxic Chemicals

N = Nongenotoxic Chemicals

Is There a Pattern in the Missed Calls?



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7 = p53-G/Tg.AC-N

8 = p53-G/TgAC-All

9 = NTP Rodent Bioassay

10 = Rat Bioassay Plus Tg.Ac-N or Trp53-G

11 = Rat Bioassay Plus RasH2-N or Trp53-G

12 = Rat Bioassay Plus Genotoxicity

Second Board of Scientific Counselors Review Results

- Pritchard et al. proposed a strategy that incorporates a rat bioassay and replaces the conventional mouse with a GMM
- Discussion points:
  - Strategy received some support, but was not “a giant step forward”
  - Discounts mouse liver carcinogens
  - GMMs provide little information on dose response
  - Mechanistic promise not yet demonstrated
  - False negatives prevent use as stand alone assay
  - Questions about potential for real cost or time savings
  - What is influence of background strain?
  - Are sufficient numbers of animals being used?
  - Is six months long enough?
  - Can data be used in risk assessment?

NTP Workshop-

- Genetically Modified Rodent Models for Cancer Hazard Identification: Selecting Substances for Study and Interpreting and Communicating Results

- February 21, 2003, Washington, DC

Workshop Charge

- Does the scientific/regulatory community consider tumor findings in genetically modified mouse models as equivalent to tumor findings in traditional rodent cancer models? Is the answer the same for all commonly used models (Tg.AC, p53+/-, rasH2)?

- To what degree is the scientific/regulatory community confident that negative results in studies with genetically modified mouse models are equivalent to negative results in the traditional bioassay?

Address these questions after working through 12 "case studies"

Workshop Outcome

- Does the scientific/regulatory community consider tumor findings in genetically modified mouse models as equivalent to tumor findings in traditional rodent cancer models? Is the answer the same for all commonly used models (Tg.AC, p53+/-, rasH2)?
  - *Answer- strong positive responses may be similar*

- To what degree is the scientific/regulatory community confident that negative results in studies with genetically modified mouse models are equivalent to negative results in the traditional bioassay?
  - *Answer- negative outcomes may not be similar- must evaluate on case by case basis*

GMM Report Foreword

The studies… designed and conducted to characterize the toxicologic potential, including carcinogenic activity, of selected agents in laboratory animals that have been genetically modified.

These genetic modifications may involve inactivation of selected tumor supressor functions or activation of oncogenes that are commonly observed in human cancers.

This may result in a rapid onset of cancer in the genetically modified animal when exposure is to agents that act directly or indirectly on the affected pathway.

An absence of a carcinogenic response may reflect either an absence of carcinogenic potential of the agent or that the selected model does not harbor the appropriate genetic modification to reduce tumor latency and allow detection of carcinogenic activity under the conditions of these subchronic studies.

Second TRR Subcommittee

- NTP Board of Scientific Counselors Technical Reports Subcommittee, May 2003
- New technical report series- GMM reports 1 and 2
  - Review of Tg.AC, p 53+/- and P16/19+/- studies of aspartame and Tg.AC and p53+/- studies of acesulfame K
- Subcommittee accepted proposed conclusions of:
  - **"*no evidence of carcinogenic activity*"** for the p 53+/-
  - **"*no evidence of positive response for papilloma formation*** in the forestomach or for tumors at other sites in male or female Tg.AC mice administered aspartame/acesulfame K in feed at concentrations up to 50,000 ppm for 9 months"
  - **"*no evidence of enhanced tumor formation in a p16/19 tumor suppressor mouse model*; this model is currently uncharacterized in terms of its expected tumor response to known rodent and/or human carcinogens and noncarcinogens"**

GMM Technical Reports

1 Aspartame

2 Acesulfame Potassium

3 Trimethylolpropane Triacrylate

4 Pentaerythritol Triacrylate

5 Bromodichloromethane

6 Sodium Bromate

7 Allyl Bromide

8 Benzene

9 Dicyclohexylcarbodiimide

10 Diisopropylcarbodiimide

11 Dichloroacetic acid

12 Phenolphthalein

13 Glycidol

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How Sensitive is the P53+/- (B6.129) Model?

- Pritchard et al. (2003) compared P53+/- results for
  - 12 known human carcinogens- identified 10
  - 19 suspected human carcinogens- identified 11
  - 28 probable human non carcinogens- identified 27
    - Overall concordance 81%
- Poor performance prompted extension of study length to 9 months for 6 additional substances
  - Aspartame (GMM 1) and acesulfame K (GMM-2)(2-year rodent non-carcinogens*- both Sal -, both positive for MN in p53+/-)
  - Sodium bromate (GMM 6) (potassium bromate was positive for kidney and mesotheliomas in rats, negative in mice- Sal +, MN +)
  - Dichloroacetic acid (GMM 11) (liver tumors rats and mice- Sal +, MN -)
  - Bromodichloromethane- (kidney, intestine tumors in rats, kidney and liver in mice- Sal +, MN -)
  - Allyl bromide (GMM 7) (allyl Cl negative in 2-year studies, Sal +, MN -)
  - <u>All were negative in the 9-month p53+/- mouse model</u>

How Sensitive is the Tg.AC Model?

- Pritchard et al. (2003) compared Tg.AC results for
  - 9 known human carcinogens- identified 8
  - 15 suspected human carcinogens- identified 9
  - 12 probable human non carcinogens- identified 10
    - Overall concordance 74%
- Poor performance prompted extension of study length to 9 months for 5 additional substances
  - Aspartame (GMM 1) and acesulfame K (GMM-2) (non carcinogens, Sal -, MN +) *both negative in Tg.AC*
  - Sodium bromate (GMM 6) (potassium bromate positive for kidney and mesotheliomas in rats, neg in mice- Sal +, MN +) *negative in Tg.AC by oral and dermal routes*
  - Dichloroacetic acid (GMM 11) (liver tumors rats and mice- Sal +, MN -) *tumorigenic in dermal and oral 9-month studies, negative at 6 months*
  - Bromodichloromethane (kidney, intestine tumors in rats, kidney and liver in mice- Sal +, MN -) *negative in 6 and 9-month studies, dermal and oral*

How Sensitive is the p16/19 +/- Model?

- Very limited database
  - 1 known human carcinogen- identified 1
  - 2 suspected human carcinogens- identified 1
  - 1 unclassifiable chemical (aspartame)- negative in model

- An extension of study length to 9 months was done for the aspartame study
  - Aspartame (GMM 1) was negative in the p16/19+/-
  - Background tumor rates were acceptably low at 9 months
  - Model is currently maintained as frozen embryos
  - No ongoing studies

Can GMMs be used in Risk Assessment?

- Majority of GMM results are not useful for risk assessment because-
  - Limited dose-response information
    - Small group sizes
    - Response almost always only at top dose

- BMDs have been calculated for TCDD from an 8-dose Tg.AC and conventional bioassays (Wyde, M, unpublished)
  - Reasonable agreement

	ED01 (ng/kg/day)
"Liver tumors" female SD rat, Kociba et al. (1978)	0.382 (0.036)
Cholangiocarcinoma female SD rat, NTP (2005)	11.33 (5.78)
Sq cell papillomas Tg.AC model Wyde et al. (2004)	0.921 (0.303)

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What is Ongoing?

- Toxic Equivalence Factor evaluation in Tg.AC mice
  - TCDD
  - PCB 126
  - PCDF
  - PCB 126/PCDF mixture

- Characterization of response of the rasH2 mouse to polybrominated diphenyl ether congeners
  - Flame retardants manufactured as crude mixtures of polybrominated diphenyl ethers
  - Known effects on thyroid function
  - rasH2 shown to respond to ethylene thiourea with thyroid adenomas
  - Limited amounts of individual congeners available for study

What is Ongoing?

- Evaluation of combination HIV therapeutics in the C3B6F1-*trp53*(+/-) mouse
  - used to prevent mother-to-child transmission of HIV
  - AZT alone shown positive in this mouse
  - AZT/3TC studies underway
  - Other possible combinations
    - AZT/3TC/NVP
    - AZT/3TC/NFV

P53+/- placed on the B6C3F1 background to provide a broader tumor susceptibility than is seen with the P53+/- on the C57BL/6 background

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Perspective

- GMM models hold promise for carcinogen identification, but
- Protocol issues, sensitivity and specificity are not yet settled
- Positive results are generally under valued
- Negative results are over valued
- The exact role and ultimate utility of GMM findings is uncertain for:
  - Report on Carcinogens
  - IARC
  - Risk assessments by regulatory agencies
- GMM models are accepted for routine use as replacements for the 2-year mouse assay only by the Center for Drug Evaluation and Research, FDA
- Promise of additional mechanistic information has not been routinely realized

Proposal for Use of GMMs in NTP Cancer Hazard Identification

- Discontinue efforts to develop and or refine GMMs intended for the study of agents for which there is little or no data available from conventional rodent bioassays
- Discontinue consideration of GMMs as a routine replacement for one or both species of rodents currently used in the conventional 2-year bioassay
- Utilize GMMs
  - when there is compelling prior evidence that suggests that the particular agent, or class of agents could be adequately studied in a particular model, or
  - when there is insufficient test agent available to employ conventional 2-yr or lifetime exposure cancer models, or
  - when studying the effects of mixtures of agents if the response of the particular model chosen is known for at least one component of the mixture